Chapter 5

PAR in the Pathogenesis of Pain in Pancreatic Disease

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1. INTRODUCTION

Pancreatitis, is a significant contributor to the "burden of gastrointestinal disease" in this country, according to a recent survey conducted by the American Gastroenterological Association (2001). In 1998 there were about 1.2 million prevalent cases, with 327,000 inpatient and 530,000 physician office visits. The estimated total direct cost for this group of diseases was $2.1 billion in 1998.

The cardinal manifestations of chronic pancreatitis are glandular (endocrine as well as exocrine insufficiency) and pain. Modern medicine has brought the former under reasonable control by replacement therapy (insulin, enzymes) but the latter continues to provide a major clinical challenge- "painful chronic pancreatitis is poorly understood and its management is controversial" (DiMagno 1999). Our lack of knowledge about what causes pain in pancreatitis has been a serious obstacle to improvement of the care of these patients, leading to various empirical approaches that are often based on purely anatomical grounds, are generally highly invasive and at best of marginal value (1998). Despite a wide variety of approaches covering innocuous (enzyme therapy), minimally invasive (endoscopic decompression, nerve blocks) and highly aggressive (surgical decompression, pancreatectomy), no consensus has emerged and no form of treatment can be considered satisfactory at the present time.

U. Lendeckel and Nigel M. Hooper (eds.), Proteases in Gastrointestinal Tissue, 123-130.

2. CURRENT THEORIES ON THE PATHOGENESIS OF PAIN IN PANCREATITIS

As with other organs, pain signaling from the pancreas involves at least three levels of neurons: the first is the primary nociceptor with its peripheral nerve endings located within the gland and its cell body located in the dorsal root ganglia. The central ends of these nociceptors terminate in the dorsal horn of the spinal cord where they make contact with neurons in the gray matter. Postsynaptic (i.e., second-order) neurons then travel cephalad within ascending pathways to synapse in several thalamic and reticular nuclei on their way to the sensory cortex. Signal transduction presents a unique challenge for nociceptors because unlike other sensory stimuli (such as light), noxious stimuli can take one of a variety of diverse forms including heat, pressure and chemical injury. In general, nociceptors convert noxious stimuli to an electrical response via specialized receptors such as the vanilloid receptor, TRPV1 and a variety of others.

A minority of patients with chronic pancreatitis and pain have readily identifiable lesions such as pseudocysts that are relatively easy to treat surgically or endoscopically. In the others, pain has traditionally been thought to result from a variety of causes including elevated intrapancreatic pressures, ischemia and fibrosis. However, it is likely that these phenomena, while clearly associated with the disease, are not the root cause of the pain. Instead, they probably are inciting factors on a background of neuronal sensitization induced by damage to the perineurium and subsequent exposure of the nerves to mediators and products of inflammation. The evidence for neuroimmune interactions in the pathogenesis of pain in humans with chronic pancreatitis has recently been reviewed (Di Sebastiano *et al* 2003). In general, the data is in keeping with evidence from the somatic literature that the persistent pain associated with peripheral tissue injury or inflammation results from long-term changes in nociceptive processing that can involve both primary sensory neurons (peripheral sensitization), as well as neurons in the spinal cord and higher structures (central sensitization). The gain of the entire system is therefore reset upwards, with the result that noxious stimuli now elicit a pain response that is much greater when compared with the normal state (hyper-algesia). A further characteristic of the sensitized state is called allodynia, a term that refers to the phenomenon in which innocuous or physiological stimuli are perceived as painful. Conceptually, one can therefore postulate that patients with pancreatic neuronal sensitization may experience mechanical allodynia: pain in response to physiological changes in intraductal pressure (which would otherwise have not been perceived). Similarly, subsequent minor flare-ups of inflammation in such patients could also cause the associated pain to be felt as severe, rather than mild (hyperalgesia).

3. A POTENTIAL ROLE FOR TRYPSIN IN PANCREATIC PAIN

Nociceptive sensitization results from both early posttranslational changes as well as later transcription-dependent changes in effector genes, with both processes occurring in the peripheral terminals of the nociceptor and in dorsal horn neurons (Woolf and Costigan 1999). In turn these processes alter the sensitivity of the system with augmentation of the response to peripheral stimuli. Many of the elements of the "inflammatory soup" described in somatic pain models (including ions (K+, H+), amines (5-HT, histamine), kinins (bradykinin), prostanoids (PGE_2), purines (ATP), cytokines (TNF, IL-1, IL-6), nitric oxide and caloric activity (heat)) are likely to result in early sensitization of pancreatic nociceptors in patients with pancreatitis as well. However, in addition to these ubiquitous elements, pancreatitis is also uniquely associated with a significant release and activation of endogenous proteases such as trypsin. Activated forms of these enzymes are detected in the pancreatic parenchyma and pancreatic juice of patients with pancreatitis (Steer 1993). Although activated enzymes are usually implicated in the pathogenesis of acute pancreatitis, they are probably also important in chronic pancreatitis, especially in the early stages. Perhaps the most conclusive evidence for the importance of the role of trypsin has come from the study of hereditary pancreatitis, which is a rare condition that leads to serial attacks of acute pancreatitis eventually followed by the development of chronic pancreatitis. Patients with hereditary pancreatitis have a mutation in the trypsinogen gene, which results in failure of cleavage and persistent tryptic activity, causing a pancreatitis that is very similar in its clinical picture to nonhereditary forms of chronic pancreatitis (Whitcomb *et al* 1996; Whitcomb 1999).

Given the importance of trypsin and other proteases in the pathogenesis of pancreatitis, we hypothesized that they may also be key players in early forms of neuronal sensitization in this condition an effect mediated by specific receptors such as the protease activated receptors (PARs), a unique family of G-protein coupled receptors (Dery and Bunnett 1999).

4. PROTEASE-ACTIVATED RECEPTORS

Proteases abound in the body in both humoral (thrombin, factor Xa) and cellular compartments (trypsin, tryptase and other tryptic enzymes from mast cells), and are capable of a wide variety of biological functions, that extend beyond simple protein degradation. They can also act as biological signals, interacting with specific receptors in the form of either traditional

receptor-ligand coupling (such as those involving the coagulation factor Xa and urokinase) or through a recently described, novel method of receptor activation, requiring proteolytic cleavage. The latter is exemplified by the interactions of thrombin, trypsin, tryptase and perhaps other serine proteases, with what are called protease (or proteinase) -activated receptors (PARs). This is a growing family of G-protein-coupled-receptors (Dery and Bunnett 1999) that are biologically unique in that they are tethered to their own ligands under resting conditions. Upon exposure to certain serine proteases (e.g. thrombin for PAR1, PAR3 and PAR4 or trypsin for PAR1, PAR-2, PAR4), this ligand is "released" by proteolytic cleavage, subsequently binding and activating the receptor, which triggers a rise in intracellular calcium via phospholipase C activation and possibly, other mechanisms. Synthetic peptides (SLIGRL in the case of rat PAR-2) corresponding to the tethered ligand can also activate the receptor without the need for proteolysis.

The original, and best studied protease activated receptor is the thrombin-sensitive PAR-1 which is thought to play a role in inflammation and cell growth(Vergnolle *et al* 1999). Much less is known about PAR-2 and its role in health and disease. However, it is expressed in a variety of gastrointestinal organs and tissues including enterocytes, pancreatic ductal epithelium, colonic and vascular smooth muscle and the enteric nervous system where it is speculated that it may be important in mediating the cytoprotective, vascular, secretory and motility responses to inflammation (Coelho *et al* 2003; Cottrell *et al* 2003; Amadesi and Bunnett 2004).

5. THE PROTEASE ACTIVATED RECEPTOR-2 AND NOCICEPTION

Recently, others and we have begun exploring a role for protease-PAR-2 signaling in primary afferent nociceptors. We first demonstrated the presence of PAR-2 mRNA and protein expression in adult rat thoracic DRG, as well as an increase in intracellular calcium in response to treatment of cultured DRG neurons with either trypsin or the PAR-2 agonist activating peptide (AcPeP) (Hoogerwerf *et al* 2001). Others have also shown that PAR-2 is expressed by a subset of peripheral nociceptive (peptidergic) neurons in the rat (Steinhoff *et al* 2000). Its activation results in the release of substance P and CGRP and the development of edema, suggesting a role for PAR-2 in the neurogenic component of inflammation. Further, PAR-2 activation can mediate both thermal and mechanical hyperalgesia (Vergnolle *et al* 2001; Kawabata *et al* 2001).

6. PAR-2 AND PANCREATIC NOCICEPTION

Studies with the specific activating peptide, as described above, convincingly demonstrate that activation of PAR-2 may play a role in nociception. Sources of proteases capable of activating PAR-2 during inflammation include leukocytes and in particular, mast cells (rich in tryptase). However, in the context of pancreatitis, trypsin is an obvious endogenous candidate ligand for PAR-2 (see discussion above) and we have begun exploring the role of this system in pancreatic pain.

The gene c-fos and its protein product fos are expressed in the spinal cord after various types of noxious stimuli, leading to its widely accepted use as a surrogate marker for nociceptive activation in response to peripheral stimulation (Harris 1998). We therefore determined whether the PAR-2 agonist, AcPep (SLIGRL-NH_2) could activate pancreatic nociceptors by studying the effect of intraductal pancreatic AcPep injections on Fos expression (Hoogerwerf *et al* 2001). AcPeP was able to directly activate pancreas-specific afferent neurons in vivo. As previously discussed noxious stimuli can either activate nociceptors or sensitize them, or both. The mechanisms and pathways involved may be separate. Sensitization is assessed by observing the effects of the proposed agent on the pain response to other forms of stimulation (chemical, mechanical or thermal in nature).

We therefore tested the sensitizing effects, if any, of PAR-2 activation on the pancreatic nociceptive response to capsaicin, a potent and noxious agonist of the TRPV1 receptor. The TRPV1 (vanilloid) receptor is a key integrator of noxious thermal and chemical stimuli expressed by nociceptive neurons (Caterina and Julius 2001). We examined PAR-2 and TRPV1 expression in dorsal root ganglia receiving innervation from the pancreas (thoracic segments, T8-T13). Ninety eight percent (259/263) of all TRPV1-IR neurons demonstrate PAR-2 IR. Conversely, 60% (259/434) of PAR-2 IR neurons also show TRPV1-IR (Hoogerwerf *et al* 2004). After intraductal injection of AcPeo and enhanced spinal Fos response to capsaicin was observed, suggesting that PAR-2 activation may sensitize the nociceptors to stimulation by capsaicin. Examination of pancreatic histology did not reveal any evidence of pancreatitis, ruling out the possibility that the Fos response was secondary to induction of inflammation in response to intraductal infusion of AcPep.

The natural agonist for PAR-2 includes trypsin and tryptase, with the former the obvious candidate in the setting of pancreatitis. We therefore tested the the effect of different doses of intraductal pancreatic trypsin injections on FOS expression was studied *in vivo* and showed that it significantly increased FOS expression over boiled trypsin in a dose-dependent manner in spinal segments receiving signals from the pancreas (Hoogerwerf *et al* 2004). We also examined whether infusion of trypsin into the pancreatic duct could provoke a behavioral pain response in awake rats. To test this we used a

surrogate assay for visceral pain, the visceromotor reflex (VMR). Acute visceral pain can cause reflex contractions of somatotopically-innervated skeletal muscle, which can be measured by electromyography (EMG). Infusion of trypsin as well as AcPep into the pancreatic duct significantly increased EMG activity of the acromiotrapezius muscle suggesting that trypsin can induce a behavioral nocisponsive effect in conscious rats. To determine whether direct activation of PAR-2 produces a similar nocisponsive effect as trypsin, the PAR-2 agonist, AcPep (1 mM), was injected into the pancreas. We examined cross de-sensitization of the nocisponsive effect to provide evidence that trypsin and PAR-2 AcPep activate the same receptor. Infusion of the pancreatic duct with AcPep significantly decreased subsequent responses to trypsin.

7. CONCLUSIONS

The studies described above have led the development of a plausible model in which PAR-2 contributes to nociceptive signaling and sensitization and may provide a novel link between inflammation and pain in pancreatitis (Figure 1). Activated trypsin in the inflamed pancreas may stimulate PAR-2 on peripheral sensory neurons, resulting in their excitation and central release of neurotransmitters such as substance P (SP) and CGRP in the spinal cord. This, in turn, can cause excitation of second-order neurons in the dorsal horn to activate ascending pathways that can relay nociceptive information to the brain. Thus, suppression of trypsin activity appears to be a legitimate target for the relief of pain in pancreatitis, independent of its effects on inflammation. If validated, these findings have major implications for the pathogenesis of pain in chronic pancreatitis and will provide novel targets for analgesic therapy.

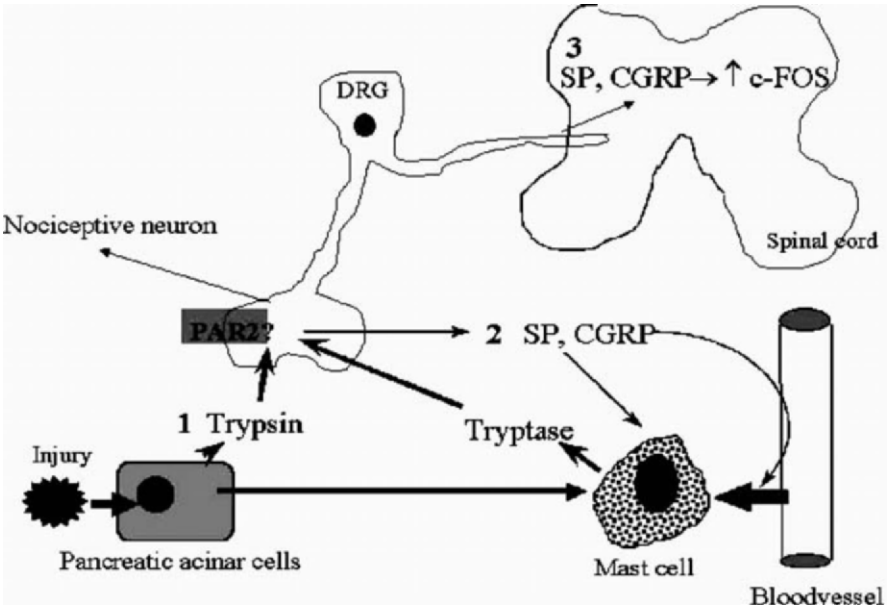

Figure 1: Proposed involvement of PAR-2 in nociceptive signaling in pancreatitis. 1. In pancreatitis, PAR-2 receptors on sensory neurons are activated by proteases such as trypsin released from injured pancreatic epithelial cells. Degranulation of mast cells releases tryptase that also acts on PAR-2 receptors. 2. PAR-2 stimulated release of CGRP and SP occurs peripherally, which further amplifies inflammation and mast cell degranulation. 3. Central release of these neurotransmitters leads to activation of nociceptivepathways and an increase in Fos expression. DRG = dorsal root ganglion. From Hoogerwerf *et al* 2001 with permission.

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